

1971 under paragraph (f)(6) of this section).

(e) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR part 792 subpart J the final test report must include the following information.

(1) *Description of system and test methods.* (i) A detailed description of the procedures used to standardize observation, including the arena and operational definitions for scoring observations.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. Historic data may be used if all aspects of the experimental protocol are the same, including personnel.

(2) *Results.* The following information must be arranged by test group dose level.

(i) In tabular form, data for each animal must be provided showing:

(A) Its identification number.

(B) Its body weight and score on each sign at each observation time, the time and cause of death (if appropriate).

(ii) Summary data for each group must include:

(A) The number of animals at the start of the test.

(B) The number of animals showing each observation score at each observation time.

(C) The percentage of animals showing each abnormal sign at each observation time.

(D) The mean and standard deviation for each continuous endpoint at each observation time.

(3) *Evaluation of data.* The findings of a functional observational battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlative histopathological findings. The evaluation shall include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any neurotoxic effects. The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

(f) *References.* For additional background information on this test guide-

line the following references should be consulted:

(1) Ankier, S.I. "New hot plate tests to quantify antinociceptive and narcotic antagonist activities," *European Journal of Pharmacology*, 27: 1-4 (1974).

(2) Coughenour, L.L., McLean, J.R. and Parker, R.B. "A new device for the rapid measurement of impaired motor function in mice," *Pharmacology, Biochemistry and Behavior*, 6: 351-353 (1977).

(3) D'Amour, F.E., Smith, D.L. "A method for determining loss of pain sensation," *Journal of Pharmacology and Experimental Therapeutics*, 72: 74-79 (1941).

(4) Deuel, R.K. "Determining sensory deficits in animals," *Methods in Psychobiology* Ed. Myers R.D. (New York: Academic Press, 1977) pp. 99-125.

(5) Edwards, P.M., Parker, V.H. "A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats," *Toxicology and Applied Pharmacology*, 40: 589-591 (1977).

(6) Evans, W.O. "A new technique for the investigation of some analgesic drugs on reflexive behavior in the rat," *Psychopharmacologia*, 2: 318-325 (1961).

(7) Irwin, S. "Comprehensive observational assessment: Ia. A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse," *Psychopharmacologia*, 13: 222-257 (1968).

(8) Marshall, J.F., Turner, B.H., Teitelbaum, P. "Sensory neglect produced by lateral hypothalamic damage," *Science*, 174: 523-525 (1971).

(9) Meyer, O.A., Tilson, H.A., Byrd, W.C., Riley, M.T. "A method for the routine assessment of fore- and hindlimb grip strength of rats and mice," *Neurobehavioral Toxicology*, 1: 233-236 (1979).

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#### § 798.6200 Motor activity.

(a) *Purpose*—(1) *General.* In the assessment and evaluation of the toxic characteristics of a substance, determination of the effects of administration of the substance on motor activity is useful when neurotoxicity is suspected.

(2) *Acute Motor Activity Test.* The purpose of the acute motor activity test is to examine changes in motor activity

occurring over a range of acute exposure levels. These changes may then be evaluated in the context of changes occurring in other organ systems. This test is an initial step in determining the potential of a substance to produce acute neurotoxicity and may be used to screen members of a class of substances for known neurotoxicity, and/or to establish a dosage regimen prior to the initiation of subchronic neurotoxicity testing.

(3) *Subchronic Motor Activity Test.* The purpose of the subchronic motor activity test is to determine whether the repeated administration of a suspected neurotoxicant results in changes in motor activity. These changes may be evaluated in the context of changes occurring in other organ systems. This test is an initial step in determining the potential of a substance to produce subchronic neurotoxicity.

(b) *Definitions.* (1) Neurotoxicity is the adverse effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

(2) Motor activity is any movement of the experimental animal.

(3) A toxic effect is an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance.

(c) *Principle of the test method.* The test substance is administered to several groups of experimental animals, one dose being used per group. Measurements of motor activity are made. The exposure levels at which significant changes in motor activity are produced are compared to those levels which produce toxic effects not originating in the central and/or peripheral nervous system.

(d) *Test procedures—(1) Animal selection—(i) Species and strain.* Testing shall be performed in a laboratory rat or mouse. The choice of species should take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, the potential for combined studies, and the availability of other toxicity data for the species.

(ii) *Age.* Young adult animals (at least 42 days old for rat or mouse) should be used.

(iii) *Sex.* (A) Equal numbers of animals of each sex are required for each dose level for the motor activity test.

(B) The females shall be nulliparous and nonpregnant.

(2) *Number of animals.* Animals shall be randomly assigned to test and control groups. Each test or control group must be designed to contain a sufficient number of animals at the completion of the study to detect a 40 percent change in activity of the test groups relative to the control group with 90 percent power at the 5 percent level. For most designs, calculations can be made according to Dixon and Massey (1957) under paragraph (f)(1) of this section, Neter and Wasserman (1974) under paragraph (f)(5) of this section, Sokal and Rohlf (1969) under paragraph (f)(9) of this section, or Jensen (1972) under paragraph (f)(3) of this section.

(3) *Control groups.* (i) A concurrent control group is required. This group must be an untreated group, or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control group are required.

(ii) Positive control data are required to demonstrate the sensitivity and reliability of the activity measuring device and testing procedure. These data should demonstrate the ability to detect increases or decreases in activity and to generate a dose-effect curve or its equivalent using three values of the dose or equivalent independent variable. A single administration of the dose (or equivalent) is sufficient. It is recommended that chemical exposure be used to collect positive control data. Positive control data shall be collected at the time of the test study unless the laboratory can demonstrate the adequacy of historical data for this purpose.

(iii) A satellite group may be treated with the high dose level for 90 days and observed for reversibility, persistence or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

(4) *Dose levels and dose selection.* At least 3 doses, equally spaced on a log scale (e.g.,  $\frac{1}{2}$  log units) over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle administration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce (A) clear effects on motor activity or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either (A) graded dose-dependent effects at 2 dose levels or (B) no effects at 2 dose levels, respectively.

(5) *Duration of testing.* The duration of exposure will be specified in the test rule.

(6) *Route of administration.* The test substance shall be administered by the method specified in the test rule. This will usually be the route most closely approximating the route of human exposure. The exposure protocol shall conform to that outlined in the appropriate acute or subchronic toxicity study guideline.

(7) *Combined protocol.* The tests described herein may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(8) *Study conduct—(i) General.* Motor activity must be monitored by an automated activity recording apparatus. The device used must be capable of detecting both increases and decreases in activity, i.e. baseline activity as measured by the device must not be so low as to preclude decreases nor so high as to preclude increases. Each device shall be tested by standard procedure to ensure, to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups must be balanced across devices. Each animal shall be tested individually. The test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for most treatments and animals. All sessions should have the same duration. Treatment groups shall be counter-balanced across test times. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables which can affect motor activity are sound level,

size and shape of the test cage, temperature, relative humidity, lighting conditions, odors, use of home cage or novel test cage and environmental distractions. Tests shall be executed by an appropriately trained individual.

(ii) *Acute.* Testing shall be timed to include the time of peak signs.

(iii) *Subchronic.* All animals shall be tested prior to initiation of exposure and at  $30 \pm 2$ ,  $60 \pm 2$  and  $90 \pm 2$  days during the exposure period. Testing shall occur prior to the daily exposure. Animals shall be weighed on each test day and at least once weekly during the exposure period.

(e) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR part 792, subpart J the final test report must include the following information:

(1) *Description of system and test methods.* (i) Positive control data from the laboratory performing the test which demonstrate the sensitivity of the procedure being used.

(ii) Procedures for calibrating and assuring the equivalence of devices and balancing treatment groups.

(2) *Results.* The following information must be arranged by test group (dose level).

(i) In tabular form, data must be provided showing for each animal:

(A) Its identification number.  
(B) Body weight, total session activity counts, and intrasession subtotals for each date measured.

(ii) Group summary data should also be reported.

(3) *Evaluation of data.* An evaluation of the test results (including statistical analysis comparing total activity counts at the end of exposure of treatment vs control animals must be made and supplied. This submission must include dose-effect curves for motor activity expressed as activity counts.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Dixon, W.J., Massey, E.J. *Introduction to Statistical Analysis* 2nd Ed. (New York: McGraw-Hill, 1957).

(2) Finger, F.W. "Measuring behavioral activity," *Methods in Psychobiology* Vol. 2. Ed. R.D. Myers (New York: Academic, 1972) pp. 1-19.

(3) Jensen, D.R. "Some simultaneous multivariate procedures using Hotelling's  $T^2$  Statistics," *Biometrics*, 28:39-53 (1972).

(4) Kinnard, E.J. and Watzman, N. "Techniques utilized in the evaluation of psychotropic drugs on animals activity," *Journal of Pharmaceutical Sciences*, 55:995-1012 (1966).

(5) Neter, J. and Wasserman, W. *Applied Linear Statistical Models*. Homewood, Richard D. Irwin, Inc., 1974.

(6) Reiter, L.E. "Use of activity measures in behavioral toxicology," *Environmental Health Perspectives*, 26:9-20 (1978).

(7) Reiter, L.W. and MacPhail, R.C. "Motor Activity: A survey of methods with potential use in toxicity testing," *Neurobehavioral Toxicology*, 1: Suppl. 1, 53-66 (1979).

(8) Robbins, T.W. "A critique of the methods available for the measurement of spontaneous motor activity," *Handbook of Psychopharmacology*. Vol. 7. Eds. Iversen, L.L., Iversen, D.S., Snyder, S.H. (New York: Plenum, 1977) pp. 37-82.

(9) Sokal, R.P. and Rohlf, E.J. *Biometry*. (San Francisco: W.H. Freeman and Co., 1969).

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#### § 798.6400 Neuropathology.

(a) *Purpose*. The techniques in this guideline are designed to develop data on morphologic changes in the nervous system for chemical substances and mixtures subject to such testing under the Toxic Substances Control Act. The data will detect and characterize morphologic changes, if and when they occur, and determine a no-effect level for such changes. Neuropathological evaluation should be complemented by other neurotoxicity studies, e.g. behavioral and neurophysiological studies. Neuropathological evaluation may be done following acute, subchronic or chronic exposure.

(b) *Definition*. Neurotoxicity or a neurotoxic effect is an adverse change in the structure or function of the nervous system following exposure to a chemical agent.

(c) *Principle of the test method*. The test substance is administered to several groups of experimental animals, one dose being used per group. The ani-

mals are sacrificed and tissues in the nervous system are examined grossly and prepared for microscopic examination. Starting with the highest dosage level, tissues are examined under the light microscope for morphologic changes, until a no effect level is determined. In cases where light microscopy has revealed neuropathology, the no effect level may be confirmed by electron microscopy.

(d) *Test procedure*—(1) *Animal selection*—(i) *Species and strain*. Testing shall be performed in the species being used in other tests for neurotoxicity. This will generally be the laboratory rat. The choice of species shall take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, the potential for combined studies, and the availability of other toxicity data for the species.

(ii) *Age*. Animals shall be young adults (150-200 gm for rats) at the start of exposure.

(iii) *Sex*. Both sexes shall be used unless it is demonstrated that one sex is refractory to the effects.

(2) *Number of animals*. A minimum of six animals per group shall be used. The tissues from each animal shall be examined separately. It is recommended that ten animals per group be used.

(3) *Control groups*. (i) A concurrent control group(s) is (are) required. This group must be an untreated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the vehicle used has a known or potential toxic property, both untreated and vehicle control groups are required.

(ii) A satellite group of animals may be treated with the high level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length; normally not less than 28 days.

(4) *Dose levels and dose selection*. At least 3 doses, equally spaced on a log scale (e.g.,  $\frac{1}{2}$  log units) over a range of